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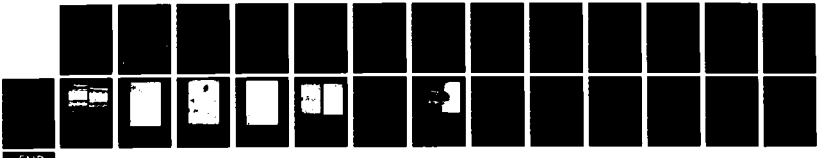

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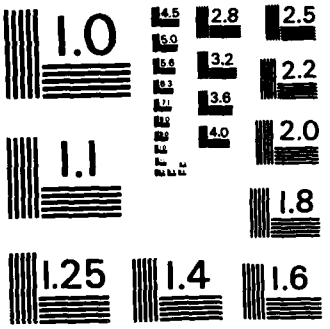

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FINAL REPORT

George Marion Hope, Ph.D.
Robert J. Ulshafer, Ph.D.

June 18, 1984

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-83-C-3066

Department of Ophthalmology
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This project was undertaken to determine the characteristics of the photoreceptor population in the retina of the bushbaby (Galago crassicaudatus), as a contribution to assessment of this nocturnal primate retina as a potential model for human scotopic, or night, vision. In particular, detection of cone (photopic) photoreceptors and, if detected,

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estimates of their frequency were primary objectives. Retinas of eight Galago were examined by light and/or electron microscopy. Additionally, retinas of a number of these animals were treated with cone-specific labels and appropriately reacted to selectively label cones. All procedures were simultaneously applied to mongoose (Herpestes auropunctatus) retinas, where cones are clearly present and easily distinguished from rods. These retinas served as a control condition, to demonstrate efficacy of the techniques applied to the experimental (Galago) retinas. Presence of cones was considered established if photoreceptors meeting four traditional structural criteria could be found and if one or more of the 'cone-specific' procedures could be shown to selectively label photoreceptors. Structures meeting two of the four anatomical criteria were identified. These were pedicle-type synaptic endings and cone-like nuclei. These are not sufficient conditions to prove presence of cones. None of the cone-specific procedures selectively labelled any photoreceptors in the Galago retinas, although all clearly selectively labelled cones in the retinas of the control species. The evidence from this study does not support the presence of cones in the Galago retina. These results indicate that the Galago retina offers significant potential as a model scotopic system.

SUMMARY

This project was undertaken to determine the characteristics of the photoreceptor population in the retina of the bushbaby (*Galago crassicaudatus*), as a contribution to assessment of this nocturnal primate retina as a potential model for human scotopic, or night, vision. In particular, detection of cone (photopic) photoreceptors and, if detected, estimates of their frequency were primary objectives. Retinas of eight *Galago* were examined by light and/or electron microscopy. Additionally, retinas of a number of these animals were treated with cone-specific labels and appropriately reacted to selectively label cones. All procedures were simultaneously applied to mongoose (*Herpestes auropunctatus*) retinas, where cones are clearly present and easily distinguished from rods. These retinas served as a control condition, to demonstrate efficacy of the techniques applied to the experimental (*Galago*) retinas. Presence of cones was considered established if photoreceptors meeting four traditional structural criteria could be found and if one or more of the 'cone-specific' procedures could be shown to selectively label photoreceptors. Structures meeting two of the four anatomical criteria were identified. These were pedicle-type synaptic endings and cone-like nuclei. These are not sufficient conditions to prove presence of cones. None of the cone-specific procedures selectively labelled any photoreceptors in the *Galago* retinas, although all clearly selectively labelled cones in the retinas of the control species. The evidence from this study does not support the presence of cones in the *Galago* retina. These results indicate that the *Galago* retina offers significant potential as a model scotopic system.

FOREWARD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the the Institute of Laboratory Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

CONTENTS

INTRODUCTION

This project was undertaken as an anatomical adjunct to physiological studies at the U. S. Army Aeromedical Research Laboratory, Ft. Rucker, investigating the potential of the bushbaby (*Galago crassicaudatus*) retina as a model for human scotopic retinal function. Ideally, for this retina to serve effectively as a model for investigation of mechanisms underlying scotopic, or night, vision, it would be desirable that the retina contain only rod photoreceptors. This would allow evaluation of scotopic mechanisms in complete isolation. The presence of cones, or photopic receptors, in small numbers would not obviate the value of the retina as a model, but an estimate of their frequency, thus potential functional contribution, would then be highly desirable.

The literature available on the photoreceptor population of the *Galago* retina is sparse and inadequately definative. Only some seven papers dealing with *Galago* photoreceptors are available (1-7). Two of these were published prior to 1940 and employed only light microscopy (1,2). Two more recent reports described the retinal anatomy of this species only superficially as emphasis was on other factors (3,4). Publications in which electron microscopy was reported dealt with the pigment epithelium (5,6) or are available only in abstract form (7). These seven reports studied four different species of *Galago* and none provided extensive photographic documentation or quantitative data. All have been in general agreement that the *Galago* retina is typically nocturnal (3), cones were not to be found (1-3) or that it contains primarily rods (7).

Physiological and behavioral studies have injected additional ambiguity into the status of this retina. Electrophysiological studies have reported a photopic ERG (8) and a Purkinje shift (shift from scotopic to photopic sensitivities) (9). On the other hand, other electrophysiological studies(3), photopigment analyses (3) and behavioral evaluations (4) have found no evidence that the photoreceptors in this retina are capable of supporting photopic vision. Departures from purely scotopic perfromance have been attributed to effects of the highly reflecting tapetum present in this species (3,4).

In summary, the anatomical studies have consistently found little or no evidence for the presence of cones in significant numbers, while functional studies have provided contradictory and somewhat ambiguous results (1-7). The present project was undertaken in an attempt to clarify the nature of the photoreceptor population(s) in the retina of *Galago crassicaudatus*, the species of interest as a potential scotopic model. Since the photoreceptors constitute the input - thus ultimate limiting - stage of the visual system, their characteristics are critical to the functional capacity of the system.

Historically, the term cone has been used to describe a

photoreceptor which has a large ellipsoid and a tapered outer segment when viewed in the light microscope (10-13). These characteristics have frequently been associated with a large nucleus, with a distinctive chromatin pattern, located distally in the outer nuclear layer, usually adjacent to the outer limiting membrane (10-13). These characteristics have traditionally been used to identify cones at the light microscopic level, and to distinguish them from the rods, which have cylindrical inner and outer segments, more proximally placed nuclei with homogeneous chromatin distribution (10-13). With the advent of electron microscopy, these classic distinctions have been found to be inadequate. Under some conditions, cones may have the appearance of rods and vice-versa. At these higher levels of magnification, however, additional features of classic cones have been found. The membrane arrangement of the cone outer segment is distinctive in that the membraneous disks are formed by infolding of the plasma membrane over the inner 30% of the outer segment (10,14). This continuity of plasma and disk membrane is present more distally in the outer segment, but with less frequency (10,14). The rod outer segments, on the other hand, consist of a stack of discrete discs completely surrounded by a plasma membrane (10,11). Additionally, the synaptic endings of cones take the general form of pedicles with high degree of synaptic complexity, whereas those of rods are more usually spherules with less synaptic complexity (10,14). In essence, if a photoreceptor is encountered which exhibits all of the characteristics of one or the other of these two sets of features, it would be considered unambiguously to fall into one of the two classes, rod or cone. However, there are many documented cases in which photoreceptors exhibit both rod and cone characteristics and classification sometimes becomes ambiguous on morphological criteria alone (10,11). Probably the only distinguishing feature for which no exceptions have been reported, and which would be most generally accepted as evidence, is the membrane configuration of the outer segment.

Within the last ten years, a number of procedures which selectively label cone photoreceptors have appeared (14-18). These include ferritin (14), procion yellow (15,16), tritiated fucose (17,18), horseradish peroxidase (17,18) and microperoxidase (17,18). Each of these procedures has been shown to have a unique affinity for one or more classes (red, blue or green) of cones in one or more species (14-18). Table 1 provides an organized display of the reported selective labelling features of these approaches.

The selectivity for specific cone types of tritiated fucose and the peroxidases has been demonstrated only for non-mammalian vertebrates (17,18). In mammals, these procedures have been reported only to label cones, without respect to type (17,18). We reasoned that employing several of these procedures, even assuming that cone type specificity generalizes to mammals, would insure that any cone type resident in the *Galago* retina would be detected. The procedures which reportedly label outer segments were viewed as most valuable since the outer segment seems, from the discussion above to be the most reliable cone identifying feature.

TABLE 1

		CONE CLASS		
TECHNIQUE	STRUCTURE	RED	GREEN	BLUE
Fucose	Outer Seg.	X		X
H.R.P.	Outer Seg.		X	
MicroP.	Outer Seg.	X		X
Proc. Yel.	Outer Seg.	X	X	X
	Entire Cell			X
Ferritin	Outer Seg.	X	X	X

The primary objectives in this study were to determine if cones are present in the retina of *Galago crassicaudatus*, and if they are, to determine relative numbers, distribution across the retina, and type classifications. We reasoned that extensive light and electron microscopy would allow detection of photoreceptors meeting a sufficient number of the anatomical criteria discussed above to justify, at least, a probability statement of the presence of cones - assuming that such photoreceptors are present. Addition of the cone specific labelling procedures, for detection purposes, would, at least, contribute to the probability statement. Then, if such photoreceptors were found, quantities of those labelled with each of the cone selective procedures could be compared to suggest the relative numbers of each cone type (red,green, blue). At the very least, if any unique (i.e., not classic rod) photoreceptors were detected, quantification of these structures would offer an estimate of their relative frequency, thus an estimate of the likelihood of deviation from purely scotopic retinal function.

METHODS

Experimental subjects were adult, male bushbabys (Galago ssicaudatus). The animals were purchased from licensed dors and were maintained in the animal care facilities of the lth center. Each animal was given vetinary medical and vetinary thalmological examinations prior to use in the study. Control nals were feral, adult, male mongooses (Herpestes opunctatus) imported from the U.S. Virgin Islands under cial permit.

The control species was included in the study to insure that selective photoreceptor labelling procedures effectively elled cone photoreceptors prior to their application to the erimental species. The mongoose was chosen for this application ause the retina of this species has been shown to contain a stantial (30 - 40%) population of easily recognizable cones, ch meet all current criteria for identification (19). onstration of the efficacy of the selective labelling procedures this species was felt to be mandatory in the event that these hniques failed to isolate cones in the Galago retinas, ce the failure could not be blamed on faulty technique.

Only two procedures were conducted on either species. First, all cases, the animals were heavily anesthetized with sodium tabarbitol by intraperitoneal and intramuscular injection. After ical anesthesia (lidocaine), the cornea of each eye was tinctively marked to preserve orientation. The animals were then her perfused through the ascending aorta with heperinized normal ine followed by cacodylate buffered (pH 7.4) aldehyde fixative gluteraldehyde + 1% paraformaldehyde) and enucleated or were cleated and euthanized by intracardiac anesthetic overdose dium pentabarbital). Second, some animals were anesthetized vily 12 or 24 hours prior to enucleation and given intravitreal ections of horseradish peroxidase, microperoxidase, or tritiated ose, in sterile, normal saline. In addition to general sthesia, liberal topical anesthesia (lidocaine) was applied to eyes and the animal was maintained under general anesthesia il reanesthesized for perfusion and/or enucleation as described.

Enucleated eyes which were uninjected and those injected with tiated fucose were immediately immersed in cold fixative. While fixative, they were opened at the ora serrata to allow free flow fixative into the globe and given additional fixation, under rigeration, overnight. The following day, the anterior segment removed and the retina was cut into small blocks for processing light and electron microscopy or, in the case of the fucose ected eyes, for autoradiography with light and electron roscopy. The retinal locus of each block was identifiable via orientation marks placed prior to enucleation and blocks were oved in an fashion to retain best orientation of receptors and er retinal components for possible quantification (20). Blocks tissue were removed in a manner such that the major meridians of retina were represented by resultant sections. The tissue was tfixed in osmium tetraoxide, dehydrated conventionally and edded in plastic (Epon 812).

Semithin (ca., 0.50 to 1.0 micron) sections were cut from this tissue and placed on glass slides and stained with toluidine blue for conventional light microscopy. Unstained slides from the fucose injected eyes were coated, in the dark, with Kodak NTB2 emulsion, exposed in the dark for 2 to 10 days and developed conventionally for light microscopic autoradiography. Ultrathin sections (ca., 500 Angstroms) were placed on copper grids and stained with uranyl acetate and lead citrate for conventional electron microscopy. Fucose treated sections were coated with Ilford L-4 emulsion, exposed for 4 to 8 weeks and developed photographically for EM autoradiography.

Eyes which had been injected with horseradish peroxidase and microperoxidase were enucleated without perfusion, as described above. These eyes were immediately opened at the ora serrata, the anterior segment removed, the retina was lightly brushed out and the optic nerve severed to release the entire structure. These retinas were then briefly (10 minutes) fixed in 1% aldehyde fixative (diluted from the standard fixative) and washed for a minimum of 24 hours in the standard buffer with 30% sucrose added. After extensive distilled water wash, the retinas were treated using Mesulum's tetramethylbenzadine procedure (21) to produce the distinctive peroxidase reaction product. After a brief dehydration, the retinas were immersed for 3 to 7 days in aged (to allow solvent evaporation), refrigerated (to inhibit polimerization) epon, under refrigeration. This procedure alows perfusion of the tissue without exposure to solvents which we have found to bleach the reaction product. The retinas were then cut into small pieces and embedded in the aged epon and allowed to air cure until polimerized (30-90 days), avoiding the heat usually employed to speed polimerization in order to improve retention of the reaction product. The cured blocks were sectioned and prepared for viewing as described above except that the sections were unstained.

Retinas to be treated for ferritin labelling were removed unperfused as described for the peroxidase treatment above. The retinas were immediately immersed in 30% ferritin in physiological saline or isotonic phosphate for 1 hour, under refrigeration. The retinas were then fixed in the standard fixative overnight under refrigeration, dehydrated and processed for viewing as described above for the unlabelled tissue, except the sections were unstained. These procedures were slightly modified from those reported by Cohen (14) for successful application of this technique.

The sections were evaluated and photographed through conventional light microscopes at magnifications from 50 to 1500 times and through conventional electron microscopes at magnifications up to 40,000 times. Structural quantification was aided by a system developed in this laboratory allowing direct computer digitization of structures viewed in the microscope or indirectly from electron micrographs, with digitized data stored on disk for computer analysis. Quantification to date has consisted of counting photoreceptor nuclei.

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Cohen, A.I. 1970. Further studies on the question of the patency of saccules in outer segments of vertebrate photoreceptors. *Vision Res. 10*: 445-453.

Laties, A.M., D. Bok and P. Liebman. 1976. Procion yellow: A marker dye for outer segment disc patency and for rod renewal. *Exp. Eye. Res. 23*: 139-148.

de Monasterio, F.M., S.J. Schein and E.P. McCrane. 1981. Staining of the blue sensitive cones of the macaque retina by a fluorescent dye. *Science 213*: 1278-1281.

Bunt, A.H. 1978. Fine structure and radioautography of rabbit photoreceptor cells. *Invest. Ophthal. Vis. Sci. 17*: 90-104.

Bunt, A.H. and I.B. Klock. 1980. Fine structure and radioautography of retinal come outer segments in goldfish and carp. *Invest. Ophthal. Vis. Sci. 19*: 707-719.

Hope, G.M., W.W. Dawson, R. Parmer, M.N. Hawthorne and D.M. Nellis. 1982. The mongoose: A potentially useful eye for retina research. *Invest. Ophthal. Vis. Sci. 22* (Suppl.): 56.

Hope, G.M. 1979. Graded differential photoreceptor orientation: Ramifications for ultramicrotomy of retina. *Stain Tech. 54*:205-211.

Mesulam, M-M. and D.L. Rosene. 1979. Sensitivity in horseradish peroxidase neurohistochemistry: A comparative and quantitative study of nine methods. *J. Histochem. Cytochem. 27*:763-773.

REFERENCES

1. Detwiler, S.R. 1939. Comparative studies upon the eyes of nocturnal lemuroids, monkeys and man. *Anat. Rec. 74*: 129-145.

2. Kolmer, W. 1930. Zur kenntnix des auges der primaten. *Zeitschr. Anat. Entwickelungsgesch. 93*: 679-722.

3. Dartnall, H.J. 1965. Anatomical, electrophysiological and pigmentary aspects of vision in the bushbaby: an interpretative study. *Vision Res. 5*: 399-424.

4. Ordy, J.M. 1968. Visual acuity and electroretinogram critical flicker frequency in relation to the morphologic organization of the retina among diurnal and nocturnal primates, squirrel monkey, marmoset, lemur, tree shrew, aotus, galago. *Vision Res. 8*: 1205-1225.

5. Braekevelt, C.R. 1980. Fine structure of the retinal epithelium in the bushbaby (*Galago senegalensis*). *Acta Anat. 107*: 276-285.

6. Frederickson, R.G. 1976. The fine structure of the choriocapillaris and retinal epithelium in the lesser bush-baby. *Anat. Rec. 184*:406.

7. Frederickson, R.G. 1975. A correlative examination of ocular structures in *galago* using transmission electron microscopy, scanning electron microscopy and light microscopy. *Anat. Rec. 181*: 375.

8. Graveline, J., M.A. Quere and J. Bert. 1967. L'electroretinogram chez un lemurien nocturne: le Galago senegalensis. *Ophthalmologica 154*: 143-150.

9. Dodt, E. 1967. Purkinje-shift in the rod eye of the bush-baby, *Galago crassicaudatus. Vision Res. 7*:509-517.

10. Cohen, A. 1972. Rods and cones. In: *Handbook of Sensory Physiology iii Physiology of Photoreceptor Organs*. M.G.F. Fuortes, Ed. Springer-Verlag, Berlin.

11. Pedler, C. 1965. Rods and cones--a fresh approach. In: *Colour Vision, Physiology and Experimental Psychology*. Ciba Symposium. Little Brown, Boston.

12. Sjöstrand, F.S. 1965. Synaptology of the retina. In: *Colour Vision, Physiology and Experimental Psychology*. Ciba Symposium. Little Brown, Boston.

13. Willmer, E.N. 1965. Duality in the retina. In: *Colour Vision, Physiology and Experimental Psychology*. Ciba Symposium. Little Brown, Boston.

xhaustively demonstrate the negative condition or prove the pposite condition - in the present case, that ALL photoreceptors in his retina are rods. Neither of these alternatives was possible ithin the temporal and budgetary limitations of this project, lthough the application of five different approaches, all of which ere demonstrably effective in a retina known to contain cones, eems to constitute a reasonable, if not exhaustive, demonstration.

It seems resonable to conclude that the photoreceptors of the etina of *Galago crassicaudatus* are probably either classic rods r are Pedler's Type B photoreceptors (11). Regardless of class, owever, the unique or questionable photoreceptors constitute only a inute fraction of the total population, less than 1.4%. Thus, this etina would appear to be unable to support significant photopic unction and would be a very reasonable model for studies of cotopic function.

DISCUSSION

In general, the results of this study do not provide convincing evidence for the existence of cones in the retina of *Galago crassicaudatus*. As discussed earlier, probably the only morphological criterion which could be considered sufficient evidence for the presence of cones would have been outer segment membrane configuration. No such outer segment configurations were found in this material. This observation was supported by the fact that the cone specific procedures failed to label any photoreceptors in the *Galago* retinas, although all except ferritin were successful in the mongoose. The only suggestion of a photoreceptor type other than traditional rods was in the observations of nuclei located at the distal boundary of the outer nuclear layer, which have a distinctive chromatin pattern, and the observation of pedicle-like synaptic endings. While both of these features are found consistently in cones, neither can be considered a *sufficient* condition for classifying a photoreceptor as such. Both Pedler (12) and Cohen (10) have described examples in which each of these features has been found to be associated with photoreceptors which are commonly recognized to be rods.

Ambiguities such as those described by Pedler (12) and Cohen (10) have resulted in several schemes of classification which avoid the rod-cone dichotomy altogether. Pedler (12) has suggested that photoreceptor classification based on the anatomy of the apparatus supporting input (outer segment) and output (synaptic ending) functions. This scheme recognizes a type A photoreceptor which has a low-sensitivity outer segment and a multichannel synaptic ending, a type B photoreceptor having a sensitive outer segment and multichannel synaptic ending, and a type C photoreceptor having a sensitive outer segment and single-channel synaptic ending (12). The type A photoreceptor corresponds closely to the traditional cone and the type C to a traditional rod. Other approaches are in general agreement that there are intermediate types between classic cones and classic rods, but may not entirely agree with Pedler's (12) classification.

In the current context, consideration of this species as a potential model for human scotopic retina, Pedler's anatomical classification system (12) has relevance. The critical feature of the retina as a scotopic model is that the photoreceptors (or the vast majority of them) must be of one of the high-sensitivity (rod-like) types, since the high-sensitivity characteristic would be consistent with scotopic function. Detection of only high-sensitivity outer segments in the regions of the unique nuclei and pedicles would suggest that these infrequent or unique photoreceptors would fall into Pedler's Type B classification (11), thus would be consistent with the hypothesis that this is a purely scotopic retina.

Caution is in order in accepting this interpretation however. Obviously, failure to detect cones DOES NOT PROVE that they are absent! The situation is a classical case of attempting to prove a negative. This, of course, is impossible. One can only

cones. This prompted a re-examination of the literature, which disclosed that reported successful application of this technique to mammalian cones was due artifactual labelling by contaminants, while more careful work has shown it to be effective in labelling only non-mammalian cones (14). This observation, coupled with the negative results of this technique in mongoose and the failure of the other labelling procedures to identify cone-like photoreceptors, suggested that the probability of obtaining useful data from extension of this approach to the bushbaby was virtually zero. Under these conditions, expenditure of these rather expensive animals seemed pointless and the ferritin procedure was not applied to the experimental species.

In summary, none of the cone-specific labelling procedures indicated the presence of cone-like photoreceptors in the bushbaby retinas, although all except one clearly and unambiguously labelled cones selectively in the cone-bearing mongoose retina. Exhaustive examination of *Galago* retinas failed to disclose conclusive evidence of cones. No cone-like inner or outer segments were found. The only evidence for cone-like photoreceptors was the infrequent appearance of cone-like nuclei and pedicle-like synaptic endings.

Quantitative evaluations were undertaken to determine the distribution of the distinctive nuclei in the two major meridians of the retina. Preliminary results of this analysis indicate that these structures are randomly scattered throughout the retina. No area of high concentration was found, even in the central retina. Numerically, the unique nuclei are seen relatively infrequently, as they constitute less than 1.4% of the outer nuclear layer. The mean spacing of the unique nuclei is 96.5 microns (SD =185.6 microns). This mean value is somewhat misleading because, as indicated by the large standard deviation, the spacing is highly variable. No distribution pattern could be discerned in the quantitative data.

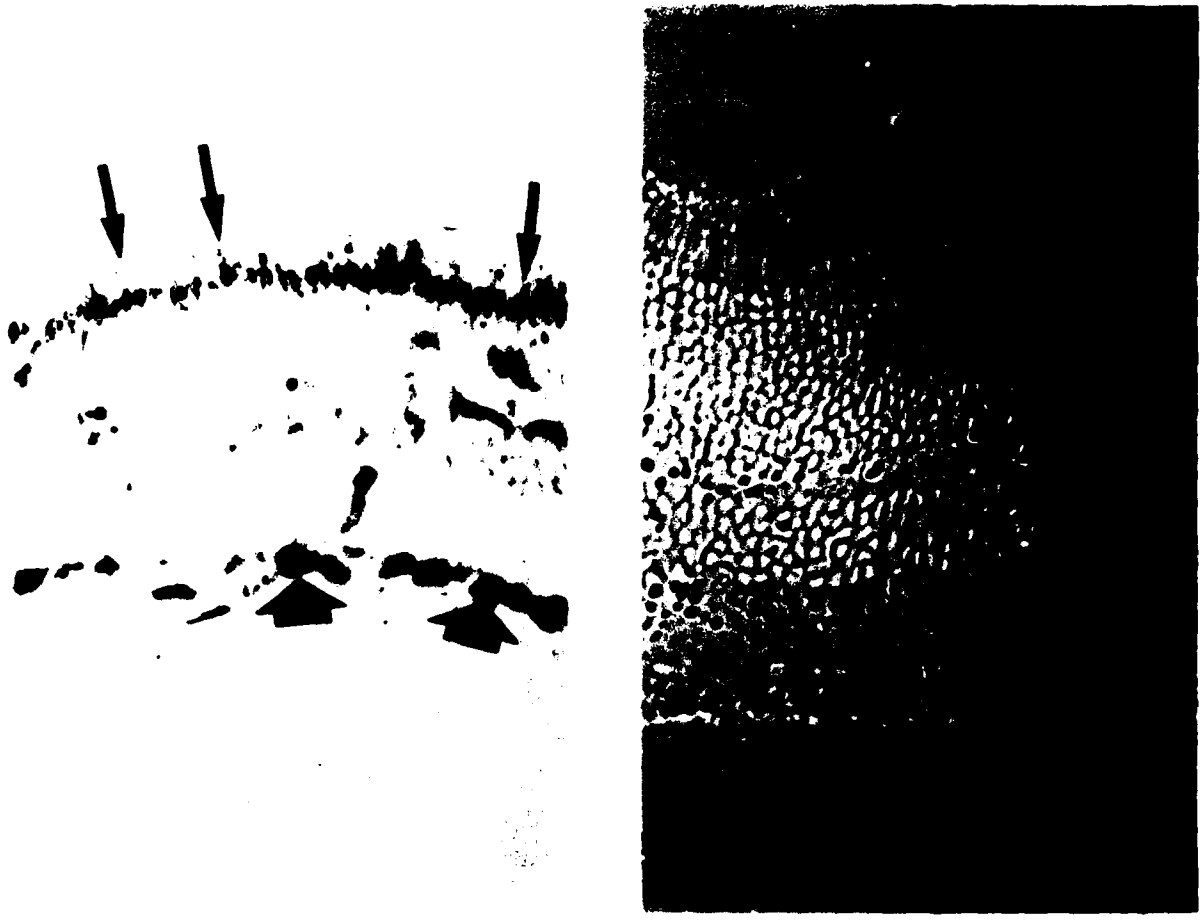

Figure 6. LIGHT MICROGRAPH OF MICROPEROXIDASE LABELLING OF CONTROL AND EXPERIMENTAL RETINAS.
The mongoose, control, retina is on the left. Note the discrete labelling of virtually every cone inner/outer segment by the microperoxidase reaction product (small arrows indicate examples). Ganglion cells also show extensive labelling (large arrows). In the bushbaby retina on the right, the photoreceptor inner/outer segment complexes (large arrow) are entirely free of microperoxidase reaction product. Reaction product can be seen in the ganglion cells (small arrows) and in two intermediate bands corresponding to synaptic regions of the outer and inner plexiform layers.

Results from the tritiated fucose procedure applied to the experimental, bushbaby retinas are illustrated in the right hand portion of Figure 5. This section has not been conventionally stained in order that the sparcity of autoradiographic grains would not be masked. Although there is substantial grain accumulation over cells of the inner retina, there is no evidence of labelling of photoreceptors. The region between the two arrows in the right margin of this photomicrograph contains the outer and inner segments of the photoreceptors, although the actual structures can not be distinguished in this unstained section. The dark material at the upper margin in this photograph is normally darkly pigmented melanine in the choroid layer. The dense band of autoradiographic grains immediately above the uppermost arrow overlies the pigmented epithelial cells, distal to the photoreceptor outer segments. A band of light grain accumulation immediately below the lower arrow overlies the outer limiting membrane and probably reflects radioactive fucose in the processes of the Muller cells. The heavy accumulations of grains in the lower portion of the micrograph are associated with cells of the inner layers of the retina. Thus, although the procedure sucessfully labelled cone outer segments in the mongoose retina, no photoreceptors were labelled in the bushbaby retinas. Labelling of other retinal cells clearly indicated that the failure to label photoreceptors was not due to technical factors.

Similar results were obtained in the peroxidase experiments. Figure 6 presents typical results of experiments in which microperoxidase was injected intravitreally 24 hours before enucleation. The cones in the mongoose retina (left micrograph) were selectively and consistently labelled (small arrows). At this magnification, the ellipsoids are most obvious. Ganglion cells containing peroxidase reaction product can be clearly seen as well (large arrows). The right-hand micrograph illustrates results of this procedure applied to the bushbaby retina. The photoreceptor inner and outer segments (large arrow) contain no reaction product. Three bands of peroxidase labelled structures can be seen and correspond to the layer of photoreceptor synaptic endings (upper band), synaptic region of the inner nuclear cells (middle band) and ganglion cells (small arrows). Both sections in this figure were unstained. As with the fucose experiments, the peroxidase technique clearly labelled cones in the control retina and non-photoreceptor structures in both retinas, but no photorecptors were labelled in the bushbaby retina.

The results of the horseradish peroxidase injections were identical to those with microperoxidase illustrated above. The HRP results are not illustrated since the micrographs are virtually identical to those in Figure 6. As with the other labelling experiments, the HRP reaction product was evident in cones of the mongoose but no photoreceptors of the bushbaby. Other cells were labelled in retinas of both species.

Results of the ferritin labelling experiments were totally negative. Extensive electron and light microscopic examination of mongoose retinas incubated in the ferritin solution failed to disclose any ferritin infiltration of interdiscal spaces of the

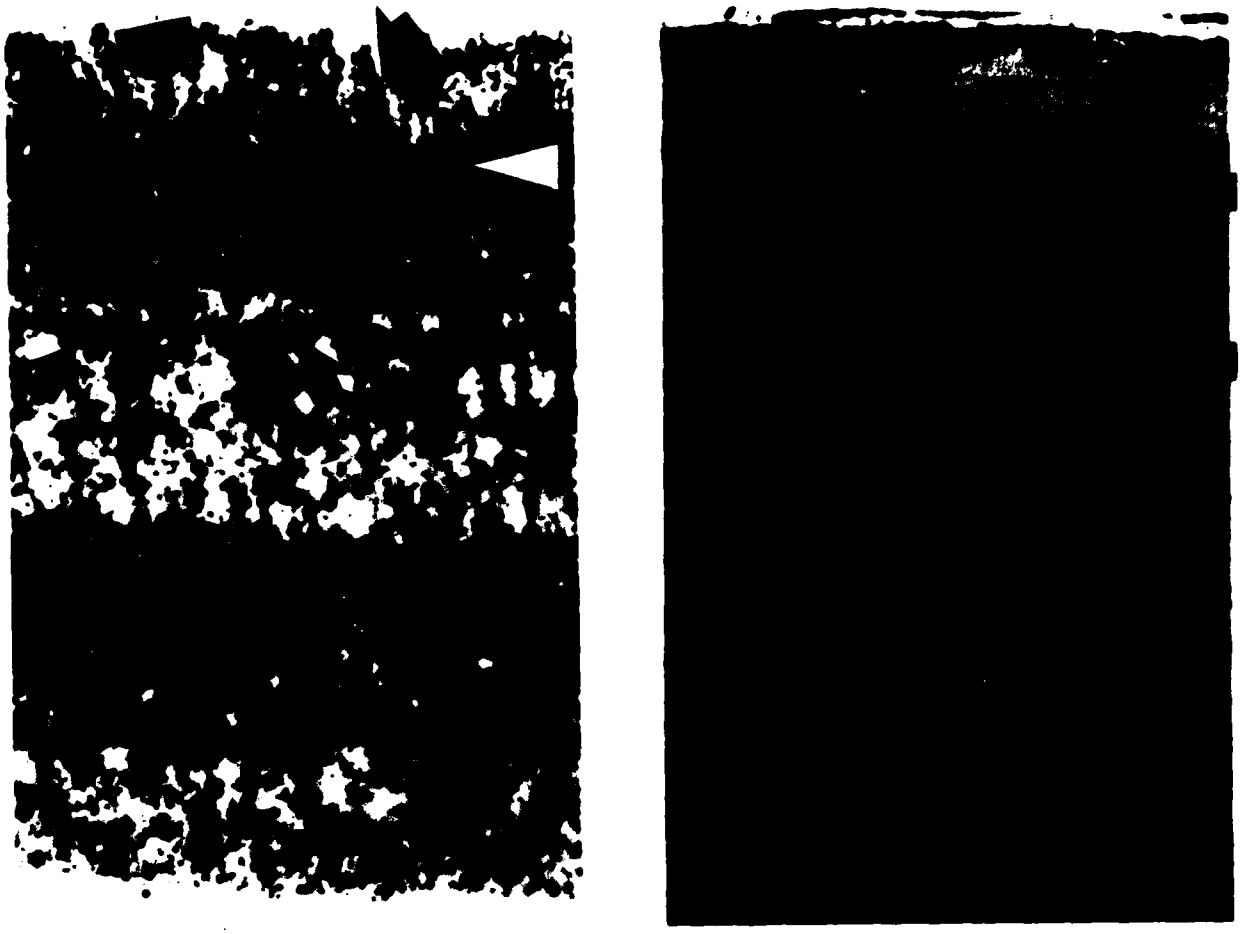

Figure 5. LIGHT MICROGRAPHS OF FUCOSE LABELLED CONTROL AND EXPERIMENTAL RETINAS.
Control, mongoose, retina is on the left. Note especially the discretely labelled cone outer segments (large arrows). The light arrow indicates dense labelling of the outer limiting membrane for reference and the small dark arrows indicate heavy labelling of photoreceptor synaptic endings. The experimental, bushbaby, retina is on the right. Note particularly the absence of label above background levels in the photoreceptor outer/inner segment region between the two arrows at the right margin. These arrows are positioned immediately below (upper arrow) the densely labelled band over the pigmented epithelial cells and above (lower arrow) the outer limiting membrane. The bottom centimeter of this photomicrograph contains only excess embedding medium with no retinal tissue, thus indicates the level of background label.

Figure 4. ELECTRON MICROGRAPH OF TYPICAL PHOTORECEPTOR INNER AND OUTER SEGMENTS OF BUSHBABY RETINA.
All photoreceptors observed were membrane bound and had only the usual small ellipsoids. Small arrows indicate plasma membranes and large arrows typical ellipsoids.

FIGURE 7. ELECTRON MICROGRAPH ILLUSTRATING PHOTORECEPTOR SYNAPTIC RIBBONS IN RUNSABY RETINA.
Ribbon like synaptic endings are indicated by large arrows. Other synaptic densities are indicated by small arrows. Note more [illegible] with contacts of the vesicles.

Figure 2. ELECTRON MICROGRAPH OF UNIQUE PHOTORECEPTOR NUCLEUS IN BUSHBABY RETINA.
A higher magnification of one of the unique nuclei is indicated by the arrow. Note the distinctive pattern of chromatin. The structure immediately right of the indicated nucleus is the apical process of a Muller cell.

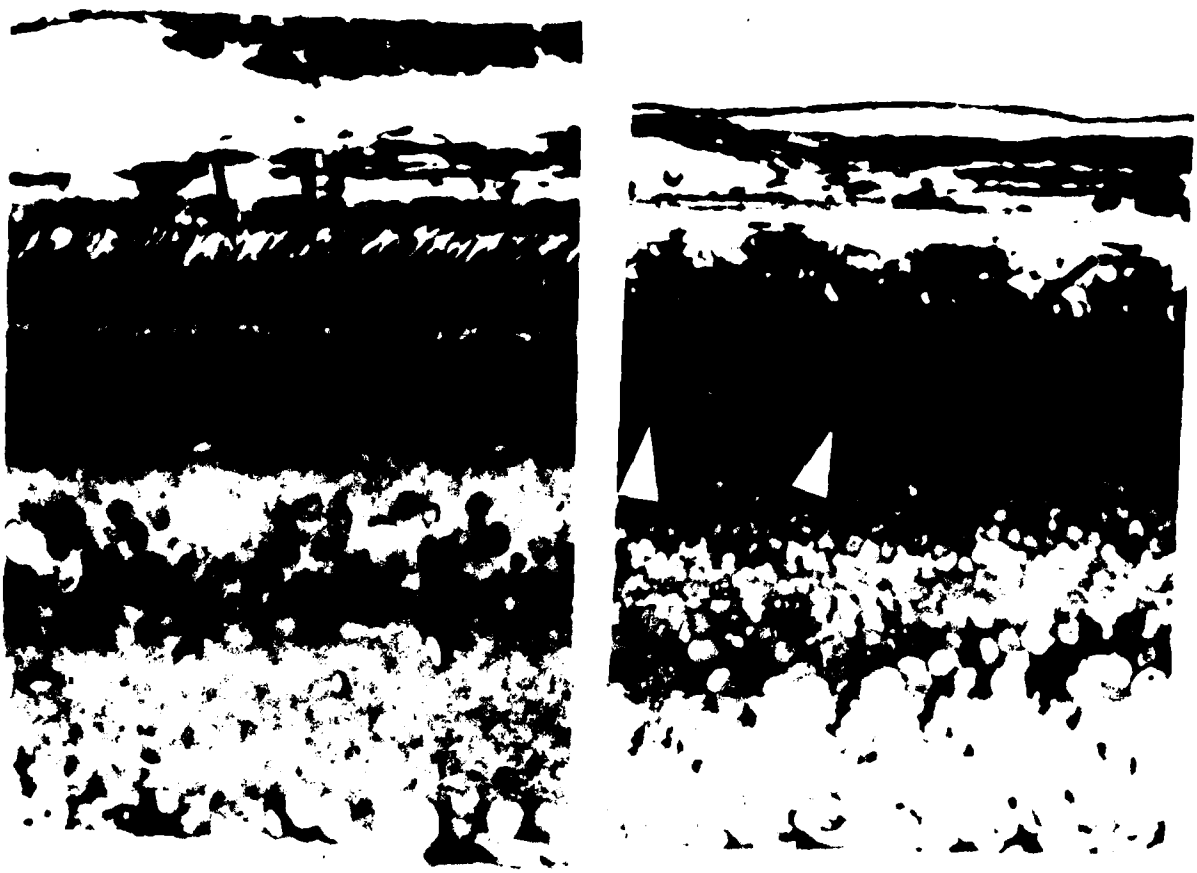

Figure 1: LIGHT MICROGRAPHS OF CONTROL AND EXPERIMENTAL RETINAS.

The retina of the mongoose, the control species is on the left. The primary feature to note is the clearly distinguishable cones (arrow). The experimental, *Galago*, retina is on the right. Note the filamentious rods (dark arrow) and unusual photoreceptor nuclei (light arrows).

RESULTS

Figure 1 presents conventional light micrographs of a control retina (mongoose) and an experimental bushbaby retina. The large cones in the mongoose retina (dark arrows) are easily identified by their large ellipsoid and tapered outer segment. The outer segments of the photoreceptors of the bushbaby retina, on the other hand are fine, filamentious structures (dark arrow) and individual ellipsoids cannot be distinguished. Two features of the outer nuclear layer (ONL) of the bushbaby retina are notable. First, the layer is notably thicker than that of the mongoose, containing many more nuclei. Second, while most of the nuclei of the bushbaby ONL are homogeneous, several distally placed, differentially stained nuclei can be seen (light arrows). These 'unique' nuclei are suggestive of the traditional 'cone' nuclei (10-13).

Electron micrographs of the bushbaby retina are presented in Figures 2-4. The unique nuclei seen in the light micrographs are evident in Figure 2 (large arrow). The apical processes of a Muller cell are immediately adjacent to the unique nucleus. These structures also stain more densely than the more common ONL nuclei and can easily be confused with unique nuclei in the light microscope. The filamentious nature of the photoreceptor outer and inner segments can be appreciated in this electron micrograph. Electron micrographic examination of the photoreceptor synaptic endings in the bushbaby retina disclosed fairly numerous pedicle-like synaptic endings (Figure 3, large arrows) with complex synaptic apparati in the basal portion. These can be compared to the smaller, simpler rod spherules (small arrows) in the area. In one instance, we were able to trace a process from one of the pedicle-like synaptic endings through the outer nuclear layer to one of the unique nuclei. Presumably therefore, the pedicle-like structures are the synaptic endings of the photoreceptors having the unique nuclei. However, we were totally unable to locate any inner-outer segment configurations which were of the type associated with cones. All outer segments in the sections containing the unique nuclei and pedicles, as well as those cut sequentially before and after, were of the conventional rod configuration, with the outer segment completely surrounded by plasma membrane (small arrows, Fig. 4) and with the typical filamentious ellipsoid (large arrows, Fig. 4). In no instance did we observe, even with exhaustive examination, an enlarged ellipsoid or an outer segment which exhibited the membrane continuities associated with cones.

Figure 5 presents typical examples of the results of the tritiated fucose experiments. The cone outer segments of the control (mongoose) retina, shown in the left-hand photomicrograph, were heavily labelled as evident by the accumulation of grains over the outer segments (large arrows). Dense bands of autoradiographic grain accumulation can be seen over the outer limiting membrane (light arrow) and over the synaptic endings of the photoreceptors (small dark arrow) Many cells of the inner retina were heavily labelled as well, as indicated by the broad band of dense grain accumulation in the bottom half of the micrograph. Conventional toluidine blue staining of this section contributes to the dense appearance.

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